



## Novel starch based nano scale enteric coatings from soybean meal for colon-specific delivery



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### ABSTRACT

Soybean meal was used to isolate resistant starch and produce nanoparticles, which could be potential coating materials for colonic nutrient and drug deliveries. The nanoparticles were in  $40 \pm 33.2$  nm ranges. These nanoparticles were stable under simulated human physiological conditions. The degrees of dissolution in both stomach and intestinal conditions were less than 30%. Furthermore, the nanoparticles were less susceptible to pancreatic enzymatic digestion (20%), which was also evidenced by the co-existence of B-type crystalline pattern. In addition to the dissolution and digestion studies in the upper gastrointestinal tract, the nanoparticles were subjected to in vitro fermentation by *Bifidobacterium brevis* and *Lactobacillus casei*. Both species showed an increase in growth and activity, while producing short chain fatty acids: acetate, propionate, and butyrate in varying amounts. Overall this study clearly demonstrated a novel method that can be used for colon-specific delivery of bioactive compounds such as drugs and nutrients.

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## 1. Introduction

Delivery of bioactive ingredients (therapeutics and nutraceuticals) to colon is a major challenge in both food and pharmaceutical applications. Most of bioactive compounds are susceptible, unless protected, to both human acidic and alkaline conditions in the gastric intestine system. Previous studies have shown that the encapsulation of bioactive compounds is a way to overcome these challenges (Nazzaro, Orlando, Fratianni, & Coppola, 2012; Dimantov, Greenberg, Kesselman, & Shimoni, 2004); commonly used materials for encapsulation are derived from natural, synthetic, and semi-synthetic sources (Nazzaro et al., 2012; Dimantov et al., 2004). Nevertheless, the success of delivering the bioactive compounds for colon depends on the type of encapsulation material.

Encapsulation is a technique to pack bioactive ingredients within a wall material to achieve targeted delivery (Nazzaro et al., 2012). The efficiency of encapsulation could be enhanced by

reducing the particle size to micro or nano scale; comparatively nanoparticles are capable of retaining the bioactive compounds until they reach the targeted site (Dimantov et al., 2004). Lipids, proteins, and polysaccharide based nanoparticles are widely used for encapsulation in colon-specific delivery (Miller, 2013). However, these particles are labile during gastric transit: materials react for pH changes in acidic ( $\text{pH} < 2$ ) and alkaline ( $\text{pH} > 7.2$ ) conditions. In addition, these materials are susceptible to pancreatic enzyme digestion, which leads to inefficient delivery of the bioactive ingredients to the colon (Dimantov et al., 2004). Therefore, lately resistant starch (RS) has become an alternative approach in encapsulation for colon-specific delivery.

Resistant starch is a fraction of starch that escapes the digestion in the upper gastro-intestinal tract and reaches the colon to exert prebiotic effect: selectively stimulates the growth and the activity of beneficial bacterial groups such as *Bifidobacteria* and *Lactobacillus* (Gibson & Roberfroid, 1995). Furthermore, the fermentation of resistant starch can produce short chain fatty acids (SCFA)—acetate, propionate, and butyrate; these fatty acids change the intestinal pH and increase bioavailability of the divalent cations (Gibson & Roberfroid, 1995). Therefore, resistant starch could be potential encapsulating materials for colon-specific delivery.

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Studies have shown resistant starch from high amylose corn starch (HACS) after hydrothermal process and retrogradation of corn starch could be used as enteric coatings for colon-specific delivery. However, lately, there is much focus on utilization of agricultural by-products for novel applications in targeted and/or controlled delivery. The materials used for this purpose should be biocompatible and generally recognized as safe (GRAS) for human applications. The soybean meal is GRAS (FDA; Chen, Liu, Zhu, Xu, & Li, 2010)—used widely (48%) in animal feed; soybean meal is a by-product of soybean oil extraction, which is a concentrated source of carbohydrates (40%). Therefore, this study was carried out to: (1) isolate resistant starch from soybean meal (2) produce and characterize the nanoparticles of isolated resistant starch (3) determine enteric stability of the nanoscale resistant starch, and (4) examine the prebiotic effects by *Bifidobacterium brevis* and *Lactobacillus casei* under in vitro conditions.

## 2. Materials and methods

### 2.1. Materials

Soybean meal was obtained from northern Crop Institute (NCI), Fargo, North Dakota. All chemicals, enzymes ( $\alpha$ -amylase from *Aspergillus oryzae* and Amyloglucosidase from *Aspergillus niger*). The *B. brevis* (ATCC 15700) and *L. casei* (ATCC 393) were obtained from Veterinary Diagnostic Lab, North Dakota State University. The anaerobic gas packs for in vitro fermentation were purchased from Hi-Media laboratory Pvt., Ltd. (Mumbai, India). All chemicals needed for the experiment were purchased from Sigma Aldrich Co (St. Louis, MO), VWR International (Radnor, PA), EMD Serono Inc. (Rockland, MA) and J. T. Baker Chemicals Co (Phillipsburg, NJ) and were used without further purifications.

### 2.2. Isolation of resistant starch from soybean meal

The isolation of starch from soybean meal was adapted from a previously reported procedure (Hilz, Lille, Poutanen, Schols, & Voragen, 2006). Soybean meal was defatted using Soxhlet extraction: 50.0 g of sample was treated with 500.0 ml hexane for 2.5 h. Defatted sample were mixed with 150.0 ml extraction buffer (50 mM ethylenediaminetetraacetic acid (EDTA), 50 mM sodium acetate, and 50 mM sodium oxalate at pH 5.2). The mixture was stirred for 60 min at 70 °C using a magnetic stirrer plate (VWR International LLC, West Chester, PA). After cooling to room temperature (25 °C), the sample was centrifuged for 15 min at 5000 rpm in a Beckman J2-HS (Beckman Coulter Inc., Brea, CA) centrifuge, with the precipitate discarded. The supernatant was mixed with ethanol to a final alcohol concentration of 70%. The sample was centrifuged again under the same conditions and the resulting precipitate was collected and dissolved in 50 mM sodium hydroxide with heating to 70 °C. Non-soluble particles were removed by filtration through Whatman filter paper number 4 (Whatman International Ltd, Maidstone, UK) and pectin was precipitated from clear solution by addition of solid barium chloride. The sample was then centrifuged for 10 min at 6000 rpm and the supernatant was mixed with ethanol to a final alcohol concentration of 70%. The sample was again centrifuged, with the precipitate air dried.

The resulted sample was subjected to enzymatic assay to isolate resistant starch. The resistant starch was isolated by a method reported by AOAC. Approximately 1.0 g of the sample was wetted using 2.0 ml of 80% ethanol. A volume of 30.0 ml thermostable  $\alpha$ -amylase (3000 U/ml) and 90.0 ml sodium acetate buffer (100 mM, pH 4.5) were added and the mixture was incubated in a water bath at 95 °C for 6 min with continuous stirring on a magnetic stirrer

(Henry Troemnor LLC, Thorofare, NJ). The sample was then placed in a 50 °C water bath after which 1.0 ml of amyloglucosidase (AMG) (350 U/ml) was added and the mixture was incubated at 50 °C for 30 min at 300 rpm on a mini vortex incubator (VWR international LLC, Radnor, PA). The solution was then centrifuged at 3000 rpm for 15 min at room temperature (25 °C). The supernatant was removed and the residue was air dried for 24 h.

### 2.3. Preparation of nanoparticles from soybean meal resistant starch

The soybean meal resistant starch was used to prepare nanoparticles by mechanical agitations. The resistant starch solution was prepared with ethanol at 1:5 (w/v). The solution was subjected to mechanical agitation by sonicating (40 kHz) using an ultra sonicator (Branson Inc, Chicago, IL) at 40 °C for 5 h.

### 2.4. Characterization of soybean resistant starch nanoparticles by scanning electron microscopy (SEM), field emission scanning electron microscopy (FESEM), and zeta potential

Imaging of nanoparticles with SEM was viewed with a JEOL JSM-6490LV scanning electron microscope (JEOL USA, Peabody, MA), with gold-palladium coating at an accelerating voltage of 15 kV. Imaging of nanoparticles with FESEM was viewed with a JEOL JSM-7600F scanning electron microscope (JEOL USA, Peabody, MA), with carbon coating at an accelerating voltage of 2.0 kV.

The zeta potential of the nanoparticles were detected with Zeta sizer (Marlwen, model of Nano- ZS) at 0.1 mg/ml concentration.

### 2.5. Particle size distribution of the nanoparticles

The Dynamic light scattering (DLS) was carried out by dissolving the nanoparticles in 50 mM sodium hydroxide solutions and subjecting them for the particle size analysis using a DLS Nicomp 380 particle sizing system (PSS, Port Richey, FL).

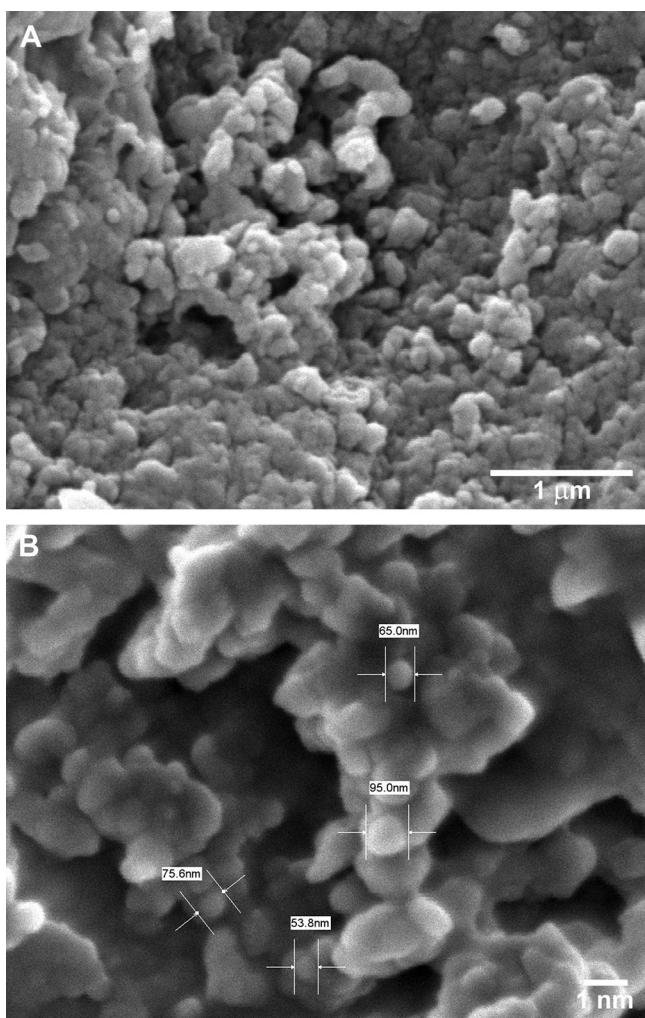
### 2.6. Dissolution tests

Dissolution studies under simulated stomach and intestine environment were adapted from Dimantov et al. (2004). For simulated stomach condition 0.1 g of nanoparticles were subjected to buffer containing 0.1 M hydrochloric acid at pH 1.5 for 3 h at 37 °C and 100 rpm. The samples were withdrawn at every 30 min time interval. For simulated intestine condition nanoparticles were subjected to buffer containing 0.1 M phosphate at pH 8.5 individually for 5 h at 37 °C and 100 rpm. The samples were withdrawn at every one hour time intervals, centrifuged at 1500 rpm and dried at 40 °C over night and weighed.

### 2.7. Digestion tests (Dimantov et al., 2004)

Digestion studies under simulated physiological conditions were adapted from Dimantov et al., 2004. Nanoparticles (0.1 g) were subjected to enzymatic solution containing pancreatic  $\alpha$ -amylase (30 U/ml) and amyloglucosidase (300 U/ml) for 16 h at 37 °C at 100 rpm. The samples were withdrawn at the end of 16 h, centrifuged at 1500 rpm and dried at 40 °C over night and weighed. The digestion was calculated as percent weight loss of the material.

Crystalline patterns were used to study the susceptibility of the soybean meal RS nanoparticles to pancreatic enzymes. Nanoparticles were subjected to XRD. X-ray powder diffraction analysis was performed using X'pert MPD (Phillip, Brussels, Belgium), operating at 40 mA and 45 kV with Cu. Freeze-dried micro scale materials



**Fig. 1.** Soybean meal resistant starch nanoparticles (a) SEM image and (b) FESEM image.

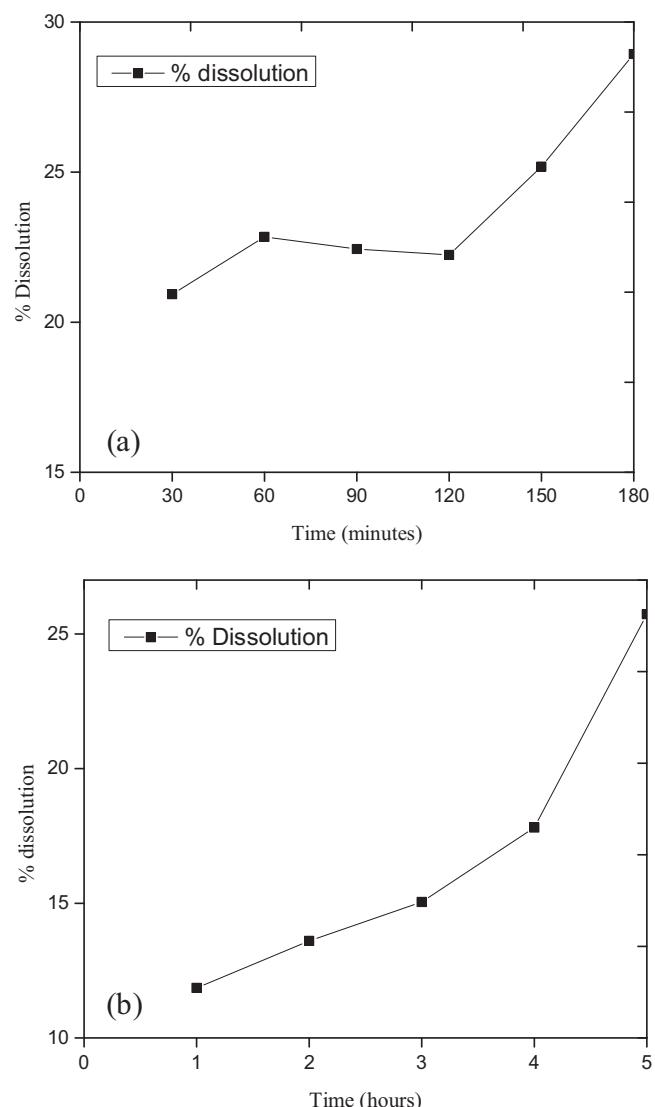
were acquired at an angular range of  $2\theta$  from  $4^\circ$  to  $99^\circ$  with step-size of  $0.05^\circ$ . Counting time was 2 s per step.

#### 2.8. In vitro fermentation of soybean meal resistant starch nanoparticles by *B. brevis* and *L. casei*

*B. brevis* and *L. casei* were maintained at  $-80^\circ\text{C}$  as glycerol stock solution. The bacteria were thawed and revived by culturing in bifidobacteria and Lactobacillus broth, respectively. The final pH of the medium was 6.8. The strains were incubated in an anaerobic incubator (VWR, Bridgeport, NJ) at  $37^\circ\text{C}$ .

#### 2.9. Growth experiments and analysis of growth characteristics

Growth experiments and analysis of growth characteristics were adapted from Zampa et al. (2004) and Wronkowska, Soral-Śmiertana, Krupa, and Biedrzycka (2006). Growth experiments were carried out in sterilize tubes. Each tube contained 3.0 ml of broth with (1% v/v) cultured cells. One milliliter of filter sterilized soybean meal resistant starch nanomaterial (5 mg/ml) was added into each tube as a carbon source. Medium with no resistant starch was used as a control. Growth was monitored by measuring the optical density using a spectrophotometer (Bio Rad smart spec 3000, Philadelphia, PA) at 600 nm ( $\text{OD}_{600}$ ) at every one



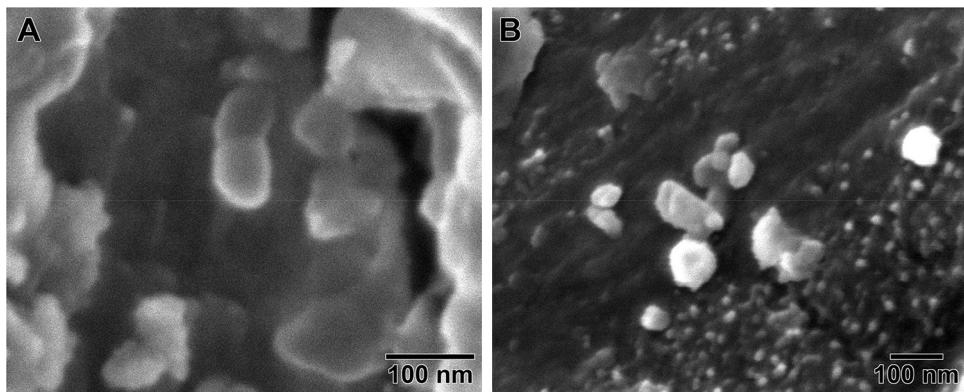
**Fig. 2.** Degree of dissolution under simulated (a) stomach and (b) intestine condition.

hour interval for 16 h (hour 0–hour 16). Growth was monitored in duplicates.

The growth was further characterized by measuring the total viable counts of the *B. brevis* and *L. casei* by spreading 10 fold dilutions of the original samples ( $10^{-1}$ – $10^{-8}$ ) on agar. The analysis was performed at every four hours including at the 0th hour (immediately after inoculation). The agar plates were spread in duplicates and were incubated in anaerobic conditions at  $37^\circ\text{C}$  for 72 h (3 days).

#### 2.10. Short chain fatty acid analysis

The samples (0.1 ml) were withdrawn from the growth media at every four hour interval over a period of 16 h. The SCFA analysis was determined by Gas Chromatography (Agilent Technology 6890N, Santa Clara, CA). A capillary column (15 m  $\times$  0.53 mm  $\times$  0.5  $\mu\text{m}$ ) was used. Helium was used as a carrier gas with a split flow rate 30.4 ml/min and a total flow rate 40.6 ml/min. The temperatures of oven, detector, and injector were maintained at  $125^\circ\text{C}$ ,  $266^\circ\text{C}$ , and  $250^\circ\text{C}$ , respectively. The peak areas were converted into their concentration as mM and mol/100 mol. The samples were analyzed in duplicates.



**Fig. 3.** FESEM images of dissolution under simulated stomach condition at pH 1.5, (A) 1 h, and (B) 3 h.

### 3. Results and discussion

#### 3.1. Nanoparticles of soybean meal resistant starch

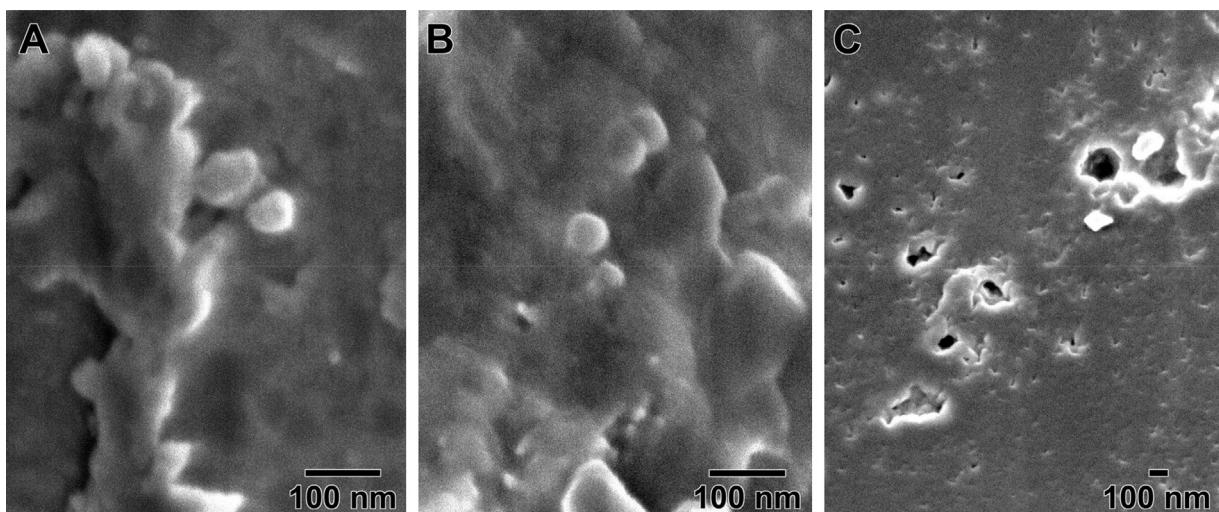
Particles with 10–1000 nm (submicron particles) of diameter are defined as nanoparticles (Parveen, Misra, & Sahoo, 2012). In this study resistant starch was isolated from soybean meal, and nanoscale particles were produced by a physical treatment—ultrasonication. The Dynamic light scattering data resulted in particle size distribution of  $40 \pm 33.2$  nm. Scanning electron microscopy (SEM) and field emission scanning electron microscopy (FESEM) in Fig. 1a and b, provides visual representation of produced nanoparticles. However, less electrostatically distorted images of FESEM showed well resolved nanoparticles (Fig. 1b). The formation of these nanoparticles could be attributed to the higher energy vibration generated from piezoelectric effect in a mechanical agitation like ultrasonication (Bel Haaj, Magnin, Pétrier, & Boufi, 2013). The high energy vibrations can lead to degradation of resistant starch granules by distorting the crystalline regions—the backbone of starch granule ((Bel Haaj et al., 2013; Gallant, Bouchet, & Baldwin, 1997)). In addition, the nanoparticles showed a zeta potential value of  $-7.69 \pm 0.46$  eV. The zeta potential values show that the nanoparticles are negatively charged; however, the lower values of nanoparticles suggest that particles can eventually aggregate. Nevertheless, the reduced particle size (to nanoscale) benefits the nanoencapsulation of bioactive compounds for controlled nutrient/drug deliveries (Nazzaro et al., 2012). Thus, the formation of

nanoparticles in this study shows the potential use of soybean meal resistant starch in nanoencapsulation—possibly as enteric coatings.

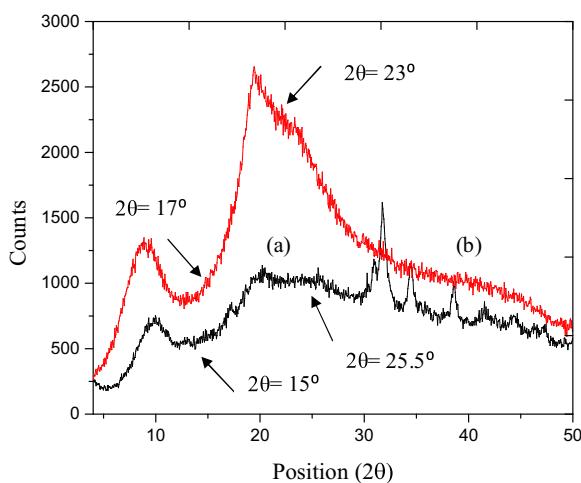
The present study yielded 1–5% of resistant-starch in soybean meal, which agrees with previously reported values (Karr-Lilenthal, Kadzere, Grieshop, & Fahey Jr, 2005). Utilization of resistant starch nanoparticles in human application provides substantial health benefits. In addition these are biocompatible—leading to reduced toxicities; however, the physiochemical stability of these nanoparticles under simulated human physiological conditions should be studied for human applications. Thus, dissolution and digestion tests of soybean meal resistant starch nanoparticles were carried out.

#### 3.2. Dissolution tests under simulated stomach and intestine conditions

The dissolution experiments were performed under simulated human stomach (pH 1.5, 3 h) and intestinal (pH 8.5, 5 h) conditions. The degrees of dissolutions were quantified as percentage weight loss—shown in Fig. 2. As depicted in Fig. 2, the degrees of dissolutions were higher under simulated stomach conditions compared to intestinal conditions at 95% confidence level. A steady increase in degrees of dissolution with time was observed under simulated intestinal condition. The increased dissolution at pH 1.5 is most likely due to the ionization of –OH group under acidic condition, which results in higher solubility. But the degree of dissolution under both stomach and intestinal conditions were less than



**Fig. 4.** FESEM images of dissolution under simulated intestine condition at pH 8.5 (A) 1 h, (B) 3 h, and (C) 5 h.



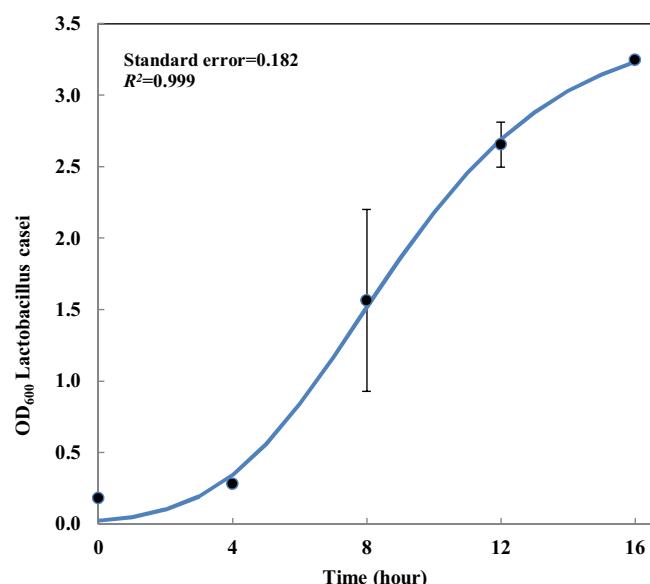
**Fig. 5.** X-ray diffraction pattern of (a) soybean meal starch; (b) soybean meal resistant starch nanoparticle.

30%; materials with less than 35% of dissolution under simulated human physiological conditions can be used as enteric coatings for colon-specific delivery (Dimantov et al., 2004). Thus, this study demonstrated the potential utilization of soybean meal resistant starch nanoparticles in colon-targeted delivery.

The degrees of dissolution were further supported by SEM images. The deformation of the nanoparticles at pH 1.5—represent the increased dissolution with time (Figs. 3 and 4). In contrast, the degree of dissolution under simulated intestine conditions did not show any significant surface changes during the first and the third hours. This is in good agreement with the quantified dissolution (Fig. 2). However, the highest dissolution at the end of 5th hour at pH 8.5 (intestine condition) is clearly seen by the formation of perforations on the surface with deformed nanoparticles. Overall observations with the quantification and surface characterization (2D) proves the stability of the nanoparticles under simulated human physiological conditions; these nanoparticles could remain intact until they reach the lower gastro intestine tract.

### 3.3. Enzymatic digestion

Enzyme susceptibility of soybean meal resistant starch nanoparticles for pancreatic enzymes was studied by subjecting the

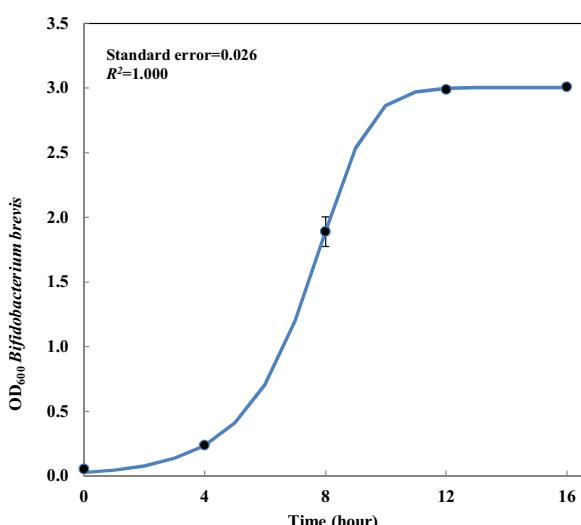


**Fig. 7.** OD<sub>600</sub> of *L. casei*.

nano particles to an enzymatic solution containing pancreatic  $\alpha$ -amylase and amyloglucosidase. The degree of enzymatic digestion was 20.3, which is more likely to occur due to the enzymatic reaction;  $\alpha$ -amylase (cleaves  $\alpha$ -1-4 linkage) and amyloglucosidase (cleaves  $\alpha$ -1-6 linkage) could penetrate into amorphous region of resistant starch-leading to enzymatic hydrolysis.

The susceptibility of the nanoparticles was further studied by X-ray diffraction patterns. The XRD patterns showed the presence of peaks at  $2\theta = 15, 17, 23$ , and  $25.5^\circ$  (Fig. 5). The peaks at  $2\theta = 15$  and  $25.5^\circ$  indicates the presence A-type crystalline pattern; whereas, the peaks at  $2\theta = 17$  and  $23$  indicates the presence of B-type crystalline patterns. The A-type crystalline patterns are susceptible to enzymatic hydrolysis compared to B-type crystalline pattern—due to the different molecular arrangements of A and B type crystalline patterns (Le, Bras, & Dufresne, 2010; Shamai, Bianco-Peled, & Shimoni, 2003). However, the co-existence of the B-type crystalline pattern in the resistant starch nanoparticles indicates that they are less susceptible to enzymatic digestion. The XRD data are in agreement with the digestion test (20.3%). These observations show the integrity of nanoparticles and demonstrate their applicability for colon-targeted delivery.

The findings so far in this study showed that nanoparticles could be produced from biocompatible and GRAS sources; these particles were stable and remained intact under simulated human physiological conditions. However, the resistant starch is a prebiotic carbohydrate: influences the growth and activity of the gut micro flora—for a pronounced gut health (Cummings, Edmond, & Magee, 2004; Murphy, Douglass, & Birkett, 2008). Thus, a further study was carried out to examine the prebiotic effect of the resistant



**Fig. 6.** OD<sub>600</sub> of *B. brevis*.

**Table 1**  
Growth of *B. brevis* and *L. casei* in terms of CFU/ml.

Time (h)	CFU/ml	
	<i>B. brevis</i>	<i>L. casei</i>
0	1.27b	1.20d
4	6.16a	2.00c
8	5.87a	5.80b
12	6.02a	6.50a
16	5.84a	6.60a
LSD ( $p < 0.05$ )	0.3641	0.2938

\* Lower transformed data; \* Each value is the mean of duplicates. Different letters on the column imply statistically significant differences at  $p < 0.05$ .

**Table 2**

Short chain fatty acid profile of *B. brevis* and *L. casei*.

Time (h)	SCFA mM					
	<i>B. brevis</i>			<i>L. casei</i>		
	Acetate	Propionate	Butyrate	Acetate	Propionate	Butyrate
0	8.30	0.40	1.00	230.71	0.00	0.00
4	28.35	0.59	1.01	226.79	0.00	0.00
8	62.97	0.55	0.36	223.31	0.00	0.00
12	125.95	0.40	0.41	203.94	0.00	0.00
16	155.32	0.42	0.43	196.18	0.00	0.00

\* Each value is a mean of duplicate.

starch nanoparticles, which could promote multiple health benefits in addition to its utilization of enteric coating for colon-targeted delivery.

### 3.4. In vitro fermentation of resistant starch nanoparticles by *B. brevis* and *L. casei*

Bifidobacteria and Lactobacillus is the most abundant gut micro flora in human. The resistant starch is one of the principle substrate for the fermentation of gut micro flora. The in vitro fermentation of *B. brevis* and *L. casei* was characterized by both the activity (optical density–OD<sub>600</sub>) and the growth (colony forming units–CFU/ml). The OD<sub>600</sub> of both *B. brevis* and *L. casei* are shown in Figs. 6 and 7, respectively. The OD<sub>600</sub> for the control (without resistant starch nanoparticles) is not reported as the values were too low. As shown in Figs. 6 and 7 the OD<sub>600</sub> was low during the first 4 h of incubation, which is most likely to occur due to the time needed for the bacteria to adjust to the new environment. After the 4th hour the OD<sub>600</sub> increased for both the species. However, the OD<sub>600</sub> reached a plateau after 12 h for *B. brevis* (Fig. 6), which is in contrast with *L. casei*. Unlike *B. brevis*, the OD<sub>600</sub> for *L. casei* kept increasing during the 16 h incubation. This observation shows that the *L. casei* utilizes resistant starch at a slower rate compared to *B. brevis*, which reached saturation after 12 h; but both *B. brevis* and *L. casei* demonstrated their ability to use resistant starch nanoparticles during 16 h.

The growth of the *B. brevis* and *L. casei* was further characterized by CFU/ml. The *B. brevis* did not show a uniform trend in growth, whereas *L. casei* showed an increase in growth (Table 1). The growth for the control was not quantified due to the absence of colony formation. At the end of the fermentation the growth of *B. brevis* increased by 4.5 folds while in *L. casei* it was 5.5 folds. Overall, the in vitro fermentation of resistant starch nanoparticles by *B. brevis* and *L. casei* proved the prebiotic effect of resistant starch, which can affect the host health and nutrition.

### 3.5. Short chain fatty acid production

Fermentation of resistant starch yields short chain fatty acids (SCFA) such as acetate, propionate, and butyrate. These are known to provide host with substantial health benefits (Gibson & Roberfroid, 1995). The SCFA provides sufficient energy necessary for the metabolism of the epithelial cells in the colon to maintain the gut health; they lead to a decrease in the lower gastro intestinal pH—to suppress the pathogenic bacteria and facilitate the mineral absorption. Thus, SCFA profile of in vitro fermentation of *B. brevis* and *L. casei* were quantified. As shown in Table 2, the acetate was produced by both *B. brevis* and *L. casei*. However, propionate and butyrate was produced only by *B. brevis*; amount of propionate and butyrate decreased with time.

Acetate, propionate, and butyrate are known to influence the human health in multiple ways. Acetate inhibits the harmful bacteria; this is utilized as a secondary fuel for skeletal and cardiac

muscles. In this study, although the acetate was formed by both species, higher amount of acetate formation was seen in *L. casei*. This observation can be attributed to the alternative homofermentation pathway: pyruvate is converted to lactic acid, formate, and acetyl CoA, which can further be converted to acetate (Klewicki & Klewicka, 2004). Homofermentation pathway is common only in Lactobacillus species, which resulted in higher acetate compared to *B. brevis*.

Propionates were observed only in *B. brevis*; Lactobacillus species does not form propionate (Klewicki & Klewicka, 2004). However, the propionate formed during the fermentation by *B. brevis* decreased with time. The decrease of propionate can be attributed to the conversion of propionate to acetate through methylmalonyl CoA pathway (Lesmes, Beards, Gibson, Tuohy, & Shimoni, 2008). In addition previous studies have reported that both bifidobacteria and Lactobacillus do not produce butyrate (Zampa et al., 2004; Gibson & Roberfroid, 1995). In contrast, we found that the butyrates were formed by *B. brevis*; however, at this point we could only speculate the possible pathway of butyrate production through butyryl CoA/acetyl CoA mediated by free acetate (Lesmes, Beards, Gibson, Tuohy, & Shimoni, 2008)—butyrates are important for ATP production, which provides 60–70% of energy needed for the colon epithelial cells (Lesmes, Beards, Gibson, Tuohy, & Shimoni, 2008; Zampa et al., 2004; Gibson & Roberfroid, 1995). Therefore, the production of SCFA (acetate, propionate, and butyrate) by *B. brevis* and *L. casei* clearly shows—the potential health benefits that could be provided—by soybean meal resistant starch nanoparticles—for enteric coatings in colon-specific delivery, while demonstrating good prebiotic effects for betterment of gut health.

### 4. Conclusion

This study isolated resistant starch from soybean meal followed by a physical treatment (ultrasonication)—to produce nanoparticles. The resulting nanoparticles were stable under simulated human physiological conditions. The results clearly showed that resistant starch nanoparticles are suitable for colon-targeted applications. In addition, these materials also showed the effects on growth and activity of gut micro flora (*B. brevis* and *L. casei*) as determined by optical density, colony forming units, and SCFA production. Future studies can be done using resistant starch nanomaterials from soybean meal for encapsulating colon-targeted bioactive compounds in medicinal and pharmaceutical chemistry.

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